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Polyoma Virus Minichromosomes: Poly ADP-Ribosylation of Associated Chromatin Proteins

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The host nuclear enzyme poly(ADP-ribose) polymerase has been shown to be associated with the replicative intermediate and mature forms of polyoma virus minichromosomes. Minichromosome-associated histones H2A and H2B as well as several nonhistone proteins were poly ADP-ribosylated by endogenous poly(ADP-ribose) polymerase. In addition, minichromosome fractions catalyzed the formation in vitro of dimers of endogenous histone H1 linked by poly(ADP-ribose). Poly ADP-ribosylated polyoma virus minichromosome chromatin labeled in vivo with [^{3}H]thymidine could be retained and eluted from anti-poly(ADP-ribose) immunoglobulin G-Sepharose. Pulse-labeled replicative intermediate minichromosomes were retained better on the antibody columns than were mature minichromosomes labeled for 2.5 h. The possible role of poly ADP-ribosylation of viral nucleosomes during polyoma replication or transcription is discussed.

The nuclear enzyme poly(ADP-ribose) [poly(ADP-rib)] polymerase alters the structure of chromatin by the generation of chains of NAD-derived poly(ADP-rib) which are covalently bound to nuclear proteins (14). The poly ADP-ribosylation of histone H1 leads to a cross-linking of this histone to form a dimeric complex (2, 6, 22, 24, 28). We have shown that this reaction leads to extensive complexation of oligonucleosomes in vitro (7). Based on these findings, it has been speculated that this natural nuclear biopolymer may be a ubiquitous cross-linking agent, effecting a transient and localized condensation of adjacent or neighboring nucleosomes to serve some function in either DNA replication or repair synthesis (2, 4, 8, 25). The minichromosome structure of polyoma virus offers a simple model system to investigate some of the yet undefined roles of poly ADP-ribosylation. In this regard, the recent report of the poly ADP-ribosylation of simian virus 40 (SV40) T antigen is of considerable relevance (10). These data suggested that papova viruses utilize this host nuclear protein modification during some stage of viral replication.

All of the enzymes and proteins for polyoma viral DNA replication are thought to be derived from the host cell, with the exception of T antigen (29). Moreover, it has been established that SV40 minichromosomal proteins are modified via kinases and acetylases, as are the host counterparts. Thus, it was of importance to determine whether poly(ADP-rib) polymerase was also a significant host-derived enzyme in replicating polyoma minichromosomes.

Using the criteria of cosedimentation with polyoma minichromosomes and affinity to poly(ADP-rib) antibody as well as direct analysis of poly(ADP-rib) acceptors, we investigated the poly ADP-ribosylation of the various forms of polyoma virus minichromosomes.

MATERIALS AND METHODS

Preparation of polyoma minichromosomes. Mouse 3T6 cells were infected as described previously by Gourlie et al. (11) with a large-plaque strain of polyoma virus grown from a single-plaque isolate at low multiplicity (10^{-3}) on baby mouse kidney cells. Infected cells were harvested at 28 h postinfection. To label replicative intermediates, viral DNA was pulse-labeled in vivo with [^{3}H]thymidine (Amersham Corp.; 55 ci/mmol) (100 μCi in 1 ml of medium) for 5 min at 37°C immediately before cell harvest. Labeling for 2.5 h at 2 μCi of [^{3}H]thymidine per ml predominately labeled mature minichromosomes. Nuclear extracts containing soluble minichromosomes were prepared from nuclei as follows. Infected cell monolayers were rinsed three times with ice-cold hypotonic buffer A (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.8], 5 mM KCl, 0.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) and drained. The cells were removed with a rubber scraper, transferred to an ice-cold Dounce homogenizer, and disrupted with 10 strokes of a tight-fitting pestle. Nuclei were pelleted at 3,000 × g in a Sorvall SS-34 rotor for 5 min. The supernatant was removed, and the nuclear pellet was suspended in buffer A (0.5 ml/15-cm plate) and extracted at 0°C for 3 h. Nuclei were removed by centrifugation (6,800 × g) for 15 min, and the supernatant solution containing soluble polyoma minichromosomes was used directly for further study.

The nuclear extract supernatant solution (1.8 ml) was layered onto a 30-ml linear sucrose gradient (5 to 20%, wt/vol) prepared in buffer A above a 2-ml cushion of 50% sucrose and was centrifuged in an SW27 rotor (Beckman Instruments, Inc.) at 25,000 rpm for 3 h at 4°C (20, 21). Fractions (1 ml) were collected from the top.

Enzyme assays. Poly(ADP-rib) polymerase activity was measured in a reaction mixture containing 50 mM Tris-hydrochloride (pH 8.0), 2 mM $MgCl_2$, 1 mM dithiothreitol, [^{32}P]NAD, and polyoma minichromosomes or HeLa chromatin fractions or both as sources of enzyme activity in a total volume of 50 μl. The reaction was carried out for 20 min at 25°C and terminated by the addition of 2 ml of 20% trichloroacetic acid–5 mM pyrophosphate. Radioactivity incorporated as insoluble material was collected on glass fiber filters (Whatman, Inc.), solubilized in 0.5 ml of Protosol (New England Nuclear Corp.), and quantitated by liquid scintillation counting.

Analysis of labeled proteins in polyacrylamide gels containing SDS. The poly ADP-ribosylation reactions were carried out with pooled fractions from the sucrose gradient as described in the legend to Fig. 4. The total proteins were precipitated with 3 volumes of absolute ethanol at −20°C for 16 h. The precipitated material was centrifuged at 10,000 × g for 10 min, dissolved in 10 mM Tris-hydrochloride (pH 6.8)–0.1% sodium dodecyl sulfate (SDS)–6 M urea–1% 2-mercaptoethanol–20% glycerol, and boiled for 2 min before electrophoresis. Electrophoresis was performed in 10% polyacrylamide (acrylamide–*N*,*N*-methylenebisacrylamide [200:1, wt/vol]) slab gels with 50 mM sodium phosphate (pH 7.0) and 0.1% SDS in the gel buffer. The gels were dried on a Bio-Rad model 224 slab gel drier and exposed for autoradiography for an appropriate period.

Autoradiography. Kodak SB-5 X-ray film was exposed to dried gels containing ^{32}P-labeled proteins with a Dupont Cronex intensifier screen at −70°C for the appropriate times.

Analysis of acid-soluble proteins on acetic acid-urea gels. Poly ADP-ribosylated proteins were extracted with 0.4 N H_2SO_4 for 30 min and precipitated with 20% trichloroacetic acid. The precipitates were washed with acetone, dried, and dissolved in a solution containing 0.9 N acetic acid, 10% sucrose, 8 M urea, and 4% 2-mercaptoethanol. Histone H1 was preferentially extracted by the procedure of Johns (15). Polyacrylamide (15%) gel electrophoresis was performed as described by Panyim and Chalkley (23).

RESULTS

Cosedimentation of poly(ADP-rib) polymerase activity with polyoma minichromosomes. Polyoma minichromosomes, prepared from cells labeled in vivo with [^{3}H]thymidine, were sedimented through a 5 to 20% sucrose gradient (Fig. 1). The thymidine incorporation data coupled with data from earlier electron microscopic studies of similar gradients (11, 19) indicated that fractions 5 to 10 represented the replicative intermediate (RI) region of the gradient, whereas mature minichromosomes sedimented in fractions 10 to 15. Fractions were subsequently assayed for poly(ADP-rib) polymerase activity by using endogenous acceptors. The bulk of the poly(ADP-rib) polymerase activity cosedimented coincidently with the peak of polyoma minichromosomes. It was of interest that a significant amount of activity also sedimented in the RI region of the gradient. Since there was only a minimal mass of chromatin in this fraction (~5% of the total) (19), the activity was better evaluated by measuring precise poly(ADP-rib) acceptors in this fraction (see below) and by using pooled samples of RIs (Table 1). The specific activity of the pooled RI chromatin fraction was 7×10^5 cpm per absorbance unit at 260 nm (A_{260}), compared with 4.1×10^5 for mature polyoma chromatin and 2.7×10^5 for a sample of HeLa nucleosomes (Table 1). The specific activity in RI minichromosomes appeared to be especially significant.

Poly(ADP-rib) acceptors on polyoma minichromosomes. Verification of poly(ADP-rib) polymerase and its association with polyoma minichromosomes was further provided by two additional methods: direct immunological determination with antibody specific to poly(ADP-rib) and analysis of acceptors. Whereas the data of Fig. 1 and Table 1 demonstrated polymerase activity, as assessed by poly(ADP-rib) incorporation into the acid-insoluble form, it was impor-

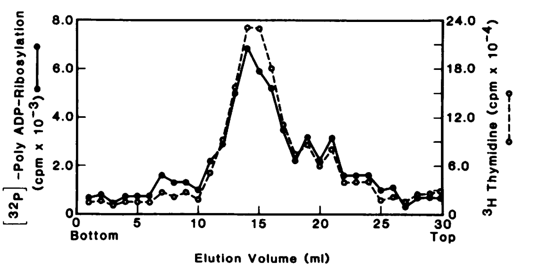

FIG. 1. Poly(ADP-rib) polymerase activity associated with polyoma minichromosomes fractionated by sucrose gradient sedimentation. A nuclear extract was applied to a 5 to 20% sucrose gradient in buffer B (12) and sedimentated at 25,000 rpm for 3 h in a Beckman SW27 rotor with a 2.0-ml cushion of 50% sucrose. Two plates of infected cells were labeled in vivo for 2.5 h with 2 μCi of [^{3}H]thymidine per ml as described in the text. [^{3}H]thymidine incorporation was monitored in 40 μl of each fraction. In addition, poly(ADP-rib) polymerase activity was assayed in 40 μl of each fraction with [^{32}P]NAD for 20 min as described in the text.

TABLE 1. Specific activities of poly(ADP-rib) polymerase of HeLa cell nucleosomes and polyoma RI and mature chromatin

Source of chromatin	Poly ADP-ribosylation[a]			Incorporation into histone fraction[b]		
	A_{260}/assay[c]	cpm ($\times 10^4$)	cpm/A_{260} ($\times 10^5$)	A_{260}/assay[c]	cpm ($\times 10^4$)	cpm/A_{260} ($\times 10^5$)
RI	0.03	2.1	7			
Mature	0.1	4.1	4.1	0.378	2.5	6.6
HeLa nucleosomes	0.2	5.3	2.7	0.409	5.2	12.7

[a] Fractions of polyoma chromatin from a gradient similar to that of Fig. 1 were pooled and assayed for poly-(ADP-rib) polymerase activity as described in the text. HeLa cell polynucleosomes (8 to 10 N) were prepared as described previously (6, 7).

[b] Polyoma and HeLa chromatin were incubated as described above. Total histones were subsequently extracted according to the method of Panyim and Chalkley (23) and counted for radioactive incorporation.

[c] The A_{260} values observed in this paper yield polyoma DNA concentrations essentially the same as those determined by Krauss and Benbow (19) by direct counting of polyoma DNA molecules from fractions of comparable sucrose gradients.

tant to ascertain whether actual covalent poly ADP-ribosylation of polyoma chromosomal proteins occurred. In other eucaryotic systems, it has been established that there are two major classes of poly(ADP-rib) acceptors, i.e., the five histones and the poly(ADP-rib) polymerase itself undergoing an automodification reaction (2, 16). In the studies described below, a preparation of HeLa cell polynucleosomes was compared with the polyoma minichromosomes for poly ADP-ribosylation of specific chromatin acceptors.

In the experiment described in Fig. 2, 0.38 A_{260} of a pooled and concentrated sample of mature polyoma minichromosomes (i.e., fractions 10 to 15 of Fig. 1) and 0.41 A_{260} of HeLa cell oligonucleosomes were incubated in vitro with [^{32}P]NAD under conditions optimal for poly ADP-ribosylation. Acid-soluble proteins from the polyoma minichromosomes were subsequently extracted with 0.4 N H_2SO_4, separated by gel electrophoresis in the acetic acid-urea system of Panyim and Chalkley (23), and exposed for autoradiography. These data (Fig. 2, lane 1) clearly show that structural components (i.e., histones) of the viral chromatin were accessible for in vitro poly ADP-ribosylation by the polymerase bound to minichromosomes. The pattern of modification of polyoma minichromosome histones H1, H2A, and H2B was qualitatively similar to that observed with HeLa cell polynucleosomes (Fig. 2, lane 2). On a nearly equal A_{260} basis, more extensive poly ADP-ribosylation of core histone and histone H1 was noted with HeLa chromatin in comparison with the polyoma preparation (Table 1). This may be due to the presence of internal single-strand breaks of nucleosomal DNA in the HeLa preparation since polymerase activity is considerably stimulated by the presence of strand breaks in DNA (2, 4, 25). A slightly higher overall specific activity for poly(ADP-rib) polymerase was noted in the polyoma system compared with the HeLa system (Table 1). This may be due to a higher accessibility of other nonhistone acceptors; however, this was not quantitated in the present study. In the polyoma preparation, histones H1, H2A, and H2B were markedly poly ADP-ribosylated in vitro. HeLa chromatin (Fig. 2, lane 2) showed additional modification of the inner core histones H3 and

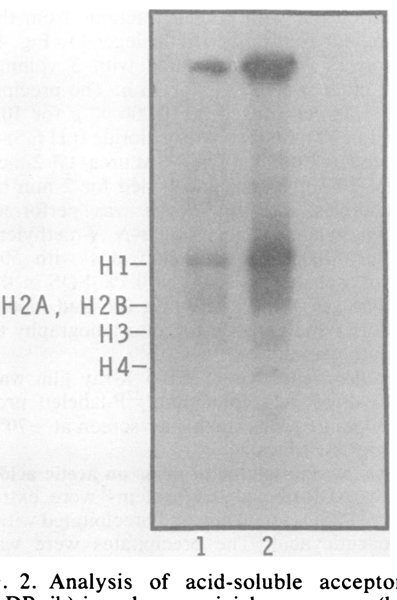

FIG. 2. Analysis of acid-soluble acceptors of poly(ADP-rib) in polyoma minichromosomes (lane 1) and HeLa chromatin (lane 2). Equivalent amounts of polyoma chromatin (0.38 A_{260}) of pooled fractions 10 to 15 of Fig. 1 or HeLa cell polynucleosomes (0.41 A_{260}) were incubated under standard conditions with 5 μCi of [^{32}P]NAD (25 nM for 5 min), and the reactions were terminated by the addition of 5 mM nicotinamide. Acid-soluble proteins were isolated and analyzed on acetic acid-urea gels as previously described (7). Autoradiography was performed as described in the text.

H4 that was not readily apparent with polyoma chromatin; however, subsequently obtained data (see below) suggest that these histones may also be modified.

Histone H1 complex formation in polyoma chromatin. One novel post-translational modification of histone H1 is the formation of a cross-linked dimer complex connected by a 15- to 16-unit chain of poly(ADP-rib) (2, 22, 27). Fewer than 5% of the potentially available H1 histones of HeLa cell chromatin appear to undergo this reaction at any one time (22). It has been speculated that this reaction may stabilize adjacent nucleosomes during some as yet unspecified functional event of chromatin (27). The syntheses of H1-poly(ADP-rib) dimer by HeLa oligonucleosomes and by polyoma minichromosomes were compared in the experiment shown in Fig. 3. In this experiment, histone H1 was selectively extracted from chromatin by the procedure of Johns (15). As established earlier, the labeling of HeLa chromatin with 12.3 μM [^{32}P]NAD results in short-chain (i.e., monomer, dimer, or trimer ADP-rib units) modification of H1 (Fig. 3, lane 3). These species of histone H1 can be subsequently "chased," by the addition of 100 μM nonradioactive NAD, via a series of intermediates (22) into the limit H1 dimer complex (Fig. 3, lane 4). A reaction similar to that found with HeLa cell nucleosomes occurred with mature polyoma minichromosomes (Fig. 3, lanes 1 and

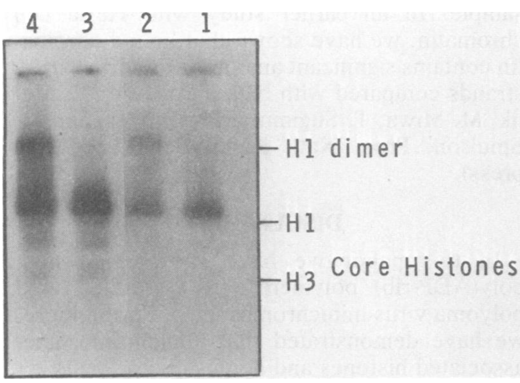

FIG. 3. Poly(ADP-rib)-histone H1 cross-linking in vitro in polyoma minichromosomes. Chromatin from polyoma (lanes 1 and 2) or HeLa cells (lanes 3 and 4) was incubated as described in the text. Each reaction was initiated by the addition of 10 μCi of [^{32}P]NAD (12.3 nM), and after 1 min at 25°C, one-half of the volume was removed (lanes 1 and 3). The other samples were chased with nonradioactive NAD at a final concentration of 100 μM for 5 min (lanes 2 and 4). The reactions were terminated with perchloric acid at a final concentration of 5% and then extracted for histone H1. Electrophoresis was performed as described by Panyim and Chalkey (23). The gel was exposed for autoradiography as described in the text.

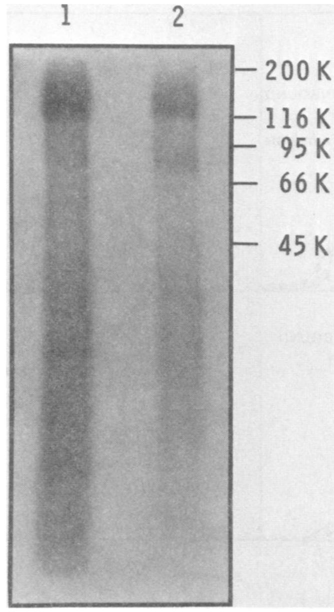

FIG. 4. SDS-polyacrylamide gel electrophoresis of in vitro nonhistone protein poly(ADP-rib) acceptors in mature and RI polyoma minichromosomes. Lanes: 1, 0.03 A_{260} of a pooled and concentrated sample of mature polyoma minichromosomes (fractions 10 to 15 of Fig. 1); 2, 0.03 A_{260} of a pooled and concentrated sample of RI polyoma minichromosomes (fractions 5 to 10 of Fig. 1). The samples were assayed and incubated in vitro under optimal conditions for poly ADP-ribosylation with 0.1 μCi of [^{32}P]NAD (25 nM). The reactions were terminated by the addition of 5 mM nicotinamide. The proteins were prepared as described in the text for SDS-polyacrylamide gel electrophoresis and autoradiography. K, Kilodaltons.

2). The data in Fig. 3 clearly demonstrate the presence of the poly ADP-ribosylated species of histone H1 in polyoma minichromosomes.

Nonhistone protein acceptors. Analysis by SDS-polyacrylamide gel electrophoresis has previously revealed that the major acceptor for poly(ADP-rib) in HeLa nuclei or nucleosomes is a high-molecular-weight nonhistone protein, of approximately 112 to 116 kilodaltons (6). This protein acceptor has been identified by Jump and Smulson (16) as poly(ADP-rib) polymerase, undergoing an automodification reaction, which can account for 50 to 80% of the enzymatic incorporation of ADP-rib. Under these conditions of ADP-ribosylation, progressively longer chains are synthesized on the nuclear protein acceptors, resulting in a retardation of mobility and a consequent lack of fine resolution (7). In the experiment shown in Fig. 4, equal A_{260} quantities of mature (lane 1) and RI (lane 2) polyoma minichromosomes were analyzed by the technique described above. Experiments

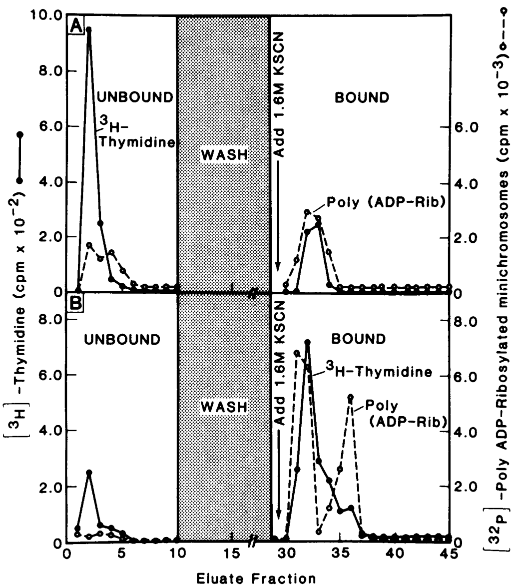

FIG. 5. Selective retention of in vitro modified polyoma chromatin by anti-poly(ADP-rib)–immunoglobulin G–Sepharose. [^{3}H]thymidine-labeled nuclear extracts were poly ADP-ribosylated with 10 μM [^{32}P]NAD (10 μCi) and passed through Sephadex G-25 as described in the text. Chromatin (1.0 ml, 1.0 A_{260}) was applied to 6 ml of anti-poly(ADP-rib)–immunoglobulin G–Sepharose 4B over 45 min as described (Malik et al., Proc. Natl. Acad. Sci. U.S.A., in press). The column was washed with phosphate-buffered saline (25 ml), and the bound material was eluted with 1.6 M KSCN. Acid-precipitable radioactivity was determined on each fraction. (A) Infected cells labeled for 5 min with [^{3}H]thymidine. (B) Infected cells labeled for 2.5 h with [^{3}H]thymidine.

previously performed with HeLa acceptors indicated that the band migrating at approximately 116 kilodaltons represents automodified poly(ADP-rib) polymerase. A second acceptor was evident at approximately 70 to 80 kilodaltons. This species was more prominent in the RI sample than in the mature chromatin sample, whereas poly(ADP-rib) polymerase automodification was more active in the latter case. Work is currently underway to characterize this species.

Partitioning of polyoma minichromosomes by a poly(ADP-rib) antibody column. In other systems, poly(ADP-rib) has been shown to be in dynamic flux with respect to synthesis and degradation (26). At any one time, only approximately 1 to 5% of the total available nucleosomal regions are accessible to modification by poly ADP-ribosylation. We have recently developed an immunoaffinity purification system using highly specific anti-poly(ADP-rib) antibodies (17). Poly ADP-ribosylated HeLa cell nucleosomes are retained by this matrix, whereas 95% of applied nucleosomes remain unbound. In the experiment shown in Fig. 5A, a polyoma nuclear extract with the DNA label distributed approximately 15% in the RIs and 85% in the mature minichromosomes was incubated with 10 μM [^{32}P]NAD and passed over the anti-poly(ADP-rib) column. An unretained fraction of labeled chromatin fractions (fractions 1 to 5) represented approximately 80% of the applied [^{3}H]DNA; some acid-insoluble [^{32}P]ADP-rib was also found in this fraction. The column was subsequently washed thoroughly with phosphate-buffered saline, and the bound, poly ADP-ribosylated polyoma chromatin was eluted with 1.6 M KSCN. Approximately 75% of the applied ^{32}P-labeled material and 10% of the [^{3}H]DNA eluted with KSCN. Previous experiments with HeLa polynucleosomes indicated that ^{32}P-labeled bound material represents poly ADP-ribosylated histones and nonhistones that are of high specific activity compared with unfractionated nucleosomes. An analogous experiment was carried out with a nuclear extract preparation containing a large proportion (80%) of the labeled DNA as RI forms of polyoma chromatin (19). The data in Fig. 5B show that the majority of the ^{32}P-labeled, poly ADP-ribosylated chromatin was retained by the immunoaffinity column. In contrast to the data of Fig. 5A, a large percentage of the [^{3}H]thymidine-labeled polyoma chromatin complex also was retained by the poly(ADP-rib) antibody in the pulse-labeled sample. In an earlier study with HeLa cell chromatin, we have shown that bound chromatin contains significant amounts of internal DNA strands compared with bulk chromatin (N. Malik, M. Miwa, T. Sugimura, P. Thraves, and M. Smulson, Proc. Natl. Acad. Sci. U.S.A., in press).

DISCUSSION

In this paper we have shown that host poly(ADP-rib) polymerase is associated with polyoma virus minichromosomes. Furthermore, we have demonstrated that minichromosome-associated histones and nonhistone proteins act as acceptors for the poly ADP-ribosylation reaction in vitro. The poly ADP-ribosylated forms of polyoma minichromosomes showed retention on a specific Sepharose column which is coupled to anti-poly(ADP-rib) antibody. This technique should, in the future, allow us to describe the potential biological consequences of the poly ADP-ribosylation with regard to polyoma viral replication.

Poly(ADP-rib) polymerase alters the structure of host chromatin by the generation of chains of NAD-derived poly(ADP-rib) which are covalently bound to various nucleosomal proteins.

Moreover, histone H1 poly ADP-ribosylation leads to a cross-linking of H1 to form a dimeric complex (27). The data in Fig. 3 show that a similar reaction occurs in vitro in the polyoma viral chromatin preparations. This observation may have important consequences for our understanding of viral replication, nucleosome assembly, or transcription, since it has been speculated that this natural bipolymer may be a ubiquitous cross-linking agent in eucaryotic chromatin. It is possible, for example, that the system for synthesizing and degrading the poly(ADP-rib) of eucaryotic chromatin effects a transient and localized condensation of chromatin by permitting a reversible interaction of adjacent or native nucleosomes within chromatin. It has been speculated that histone H1 may be required to maintain the condensed state of the SV40 minichromosomal system (9, 18, 30). In this regard it is of interest that Keller et al. (18) noted the progressive reduction in the sedimentation rate of SV40 minichromosomes upon removal of histone H1 from the complexes by raising the ionic strength.

A major question, still unanswered, is how the poly ADP-ribosylation reaction functions during the processes of polyoma replication or transcription. In this regard, more recent observations on the poly(ADP-rib) system might be relevant. It has been shown by a number of investigators, using a variety of DNA strand-breaking agents, that either in vivo or in vitro polymerase activity increases upon damage insult to DNA (13, 25). The polymerase possesses a strict requirement for DNA activity. Benjamin and Gill (3) have shown that the activity of purified poly(ADP-rib) polymerase is totally dependent upon the number of single-strand breaks within the DNA. It has been speculated that this specificity of the poly(ADP-rib) polymerase reaction might relate to its possible function in the modification of chromatin during DNA repair or, alternatively, during DNA replicative cellular events. Poly(ADP-rib) activity may play a similar role in polyoma chromatin assembly or replication. Previously we showed that DNA polymerase α activity was enriched 10-fold per molecule in the RI region of a sucrose density gradient relative to the corresponding region of the gradient for mature minichromosomes (12). In the present work, we found that the RI forms of polyoma minichromosomes have a higher specific activity for the poly ADP-ribosylation reaction than do the mature forms of the minichromosome (Table 1). The potential content of DNA single-strand breaks in the RI region of the chromatin could conceivably account for the higher activity noted in these experiments and also for the higher percentage of pulse-labeled DNA bound to the anti-poly(ADP-rib) antibody column (Fig. 5B). This conclusion is consistent with the susceptibility of polyoma RI minichromosomes to nicking at the replication fork during in vitro DNA synthetic reactions (11a).

Evidence for a second role for the poly ADP-ribosylation reaction which may relate to polyoma chromatin organization was the observation that the incubation of HeLa oligonucleosomes with NAD (at 10 μM and higher) reduced the mobility of the modified chromatin components on native chromatin-polyacrylamide gels without affecting the electrophoretic properties of the bulk nucleosomes. A direct correlation has been noted among the NAD concentration, the level of chromatin aggregation, and the length of poly(ADP-rib) chains (7). Adamietz et al. have also presented evidence indicating a potential general cross-linking of nuclear proteins via long-chain poly ADP-ribosylation (1, 2). These data emphasize a possible relationship between condensed forms of polyoma minichromosomes and a potential cross-linking or condensing function mediated by poly ADP-ribosylation. In this regard, we wonder whether the recently observed poly ADP-ribosylation of the SV40 T antigen (10) implies that it might also be cross-linked to the nucleosomal histones of the SV40 minichromosome during some stage of viral replication or transcription. Suggestive evidence has also been obtained for poly ADP-ribosylation of polyoma T antigen bound to the nuclear matrix (5). Knowledge of the roles of such modification in regulating the function or specificity of the T antigen must await more detailed studies. The well-characterized polyoma minichromosome form should provide a very useful model for further studies of the function of the poly ADP-ribosylation reaction. Furthermore, the use of immunoaffinity chromatography with the poly(ADP-rib) antibody should, in turn, allow a better appreciation of selected steps in the organization of viral minichromosomes during replication or transcription.

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